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# *Paralepistopsis* gen. nov. and *Paralepista* (*Basidiomycota, Agaricales*)

Alfredo Vizzini* & Enrico Ercole

*Dipartimento di Scienze della Vita e Biologia dei Sistemi - Università degli Studi di Torino, Viale Mattioli 25, I-10125, Torino, Italy*

*Correspondence to: *alfredo.vizzini@unito.it*

Abstract — *Paralepistopsis*, a new genus in *Agaricales*, is proposed for the rare toxic species, *Clitocybe amoenolens* from North Africa (Morocco) and southern and southwestern Europe and *C. acromelalga* from Asia (Japan and South Korea). *Paralepistopsis* is distinguished from its allied clitocyboid genera by a *Lepista flaccida*-like habit, a pileipellis with diverticulate hyphae, small non-lacrymoid basidiospores with a smooth slightly cyanophilous and inamyloid wall, and the presence of toxic acromelic acids. Combined ITS-LSU sequence analyses place *Paralepistopsis* close to *Cleistocybe* and *Catathelasma* within the tricholomatoid clade. Our phylogenetic analysis further supports *Lepista* subg. *Paralepista* (= *Lepista* sect. *Gilva*) as an independent clitocyboid evolutionary line. We recognize the genus *Paralepista*, for which we propose twelve new combinations.

Key words — *Agaricomycetes,* erythromelalgia/acromelalgic syndrome*, Clitocybe* sect. *Gilvaoideae*, /catathelasma clade

## Introduction

The genus *Clitocybe* (Fr.) Staude traditionally encompassed saprobic agarics that produce fleshy basidiomata with often adnate-decurrent lamellae, convex to funnel-shaped pilei, usually a whitish to pinkish yellow spore print, and smooth non-amyloid basidiospores (Kühner 1980, Singer 1986, Bas 1990, Raithelhuber 1995, 2004).

Recent molecular studies that included a significant number of *Clitocybe* species (Moncalvo et. al. 2002, Redhead et al. 2002, Matheny et al. 2006, Vizzini et al. 2010a,b, 2011) have shown that taxa in this traditional genus do not form a monophyletic group but rather a heterogeneous artificial set of disparate and (in many cases) phylogenetically unrelated taxa (the so-called clitocyboid fungi or *Clitocybe* s.l.).

*Clitocybe amoenolens* is a rare and rather localized species known thus far only from Morocco, southern France, northern and central Spain, and central

Italy (Malençon & Bertault 1975, Bon 1987, Poumarat & Neville 1993, Contu et al. 1999, Moreau et al. 2001, Martínez et al. 2010). It was responsible, first in France (Fourré 1997, Charignon & Garcin 1998, Moreau et al. 2001, Saviuc et al. 2001, 2002) and then in Italy (Leonardi et al. 2002, Marinetti & Recchia 2005), for induced erythromelalgia (= acromelalgia syndrome sensu Saviuc et al. 2001), a poisoning syndrome caused by the ingestion of *C. acromelalga* in Japan (Nakamura et al. 1987). This syndrome is characterized by varying degrees of tingling sensations, followed by intense burning pain in the extremities but predominantly in the feet (Saviuc & Danel 2006). *Clitocybe amoenolens* was confused with edible mushrooms in the *Lepista flaccida* complex (e.g., *L. flaccida* (Sowerby) Pat., *L. lentiginosa* (Fr.) Bresinsky, *L. gilva* (Pers.) Pat.) and with *Infundibulicybe gibba* (Pers.) Harmaja (Fourré 1997, Moreau et al. 2001).

*Clitocybe amoenolens* shows features intermediate between *Clitocybe* s.s. (smooth spores) and *Lepista* subg. *Paralepista* (cream spore-print, spotted pileus, lamellae separable from context, and cyanophilic spores released in tetrads), making its generic position uncertain.

Using recent French and Italian collections of *C. amoenolens*, we investigated its phylogenetic position within the clitocyboid fungi through morphological and molecular analyses and expanded its known geographic distribution.

## Materials & methods

### Morphology

Macromorphological features were described from fresh specimens. Microscopical preparations from dried material were rehydrated in 3% KOH and stained in Congo red, Cresyl Blue, Cotton Blue and Melzer's reagent.

Basidiospore measurements are based on means of 120 spores from prints (four collections), stained in Melzer's reagent. The basidia width was measured at the widest part and the length from the apex (sterigmata excluded) to basal septum. The following abbreviations are used in text: L = number of entire lamellae; l = number of lamellulae between each pair of entire lamellae; Q = the quotient of length and width of the spores in side view; Qm = average quotient. Colour terms in capital letters (e.g. Pale Ochraceous-Buff) are those of Ridgway (1912). Herbarium acronyms follow Thiers (2011). Author citations follow Index Fungorum (http://www.indexfungorum.org/authorsoffungalnames.htm). All examined collections are housed at TO. The new genus and new combinations are deposited in MycoBank (http://www.mycobank.org/DefaultPage.aspx ).

### DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was isolated from 1 mg of herbarium material (Table 1) using the DNeasy Plant Mini Kit (Qiagen, Milan Italy) according to the manufacturer's instructions. Universal primers ITS1F/ITS4 were used for the ITS region amplification (White et al. 1990, Gardes & Bruns 1993) and primers LR0R/LR7 for the LSU rDNA amplification (Vilgalys & Hester 1990, Vilgalys lab unpubl., http://www.botany.duke.edu/fungi/

Table 1. Collections newly sequenced in this study.

| Species | GenBank acc. numbers | | Source, country, date, collector |
|---|---|---|---|
| | ITS | LSU | |
| *Paralepistopsis amoenolens* 1 | JQ585653 | JQ585654 | TO AV2004, FRANCE, 02/09/2011, G. Moretto |
| *Paralepistopsis amoenolens* 2 | JQ585655 | — | TO AV2007, ITALY, 12/11/2011, S. Anselmino |
| *Paralepista flaccida* 1 | JQ585656 | JQ585657 | TO AV2008, ITALY, 02/09/2011, G. Moretto |
| *Paralepista flaccida* 2 | JQ585658 | JQ585659 | TO AV2009, ITALY, 20/10/2011, A. Vizzini |
| *Paralepista gilva* | JQ585660 | JQ585661 | TO AV2010, ITALY, 09/11/2011, A. Vizzini |

mycolab). Amplification reactions were performed in a PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) following Vizzini et al. (2010b). PCR products were purified with the AMPure XP kit (Beckman) and sequenced by DiNAMYCODE srl (Turin, Italy) and MACROGEN Inc. (Seoul, Republic of Korea). Sequences were assembled and edited with the phred/phrap/consed software suite. The sequences were submitted to GenBank and their accession numbers are reported in Table 1 and Figure 1.

**Sequence alignment and phylogenetic analysis**

The sequences obtained in this study were checked and assembled using Geneious v5.3 (Drummond et al. 2010) and compared to those available in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) using the blastn algorithm. Based on the blastn results, sequences were selected according to the outcomes of recent phylogenetic studies on *Agaricales* (Matheny et al. 2006, Binder et al. 2010, Vizzini et al. 2011). A combined ITS and LSU sequence analysis was carried out using sequences from the same strain or specimen. *Xeromphalina campanella* (GU320006 and GU320009) was used as outgroup. Alignments were generated using MAFFT (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. The sequence alignment, its manual adjustment, and the best-fit models estimation follow Vizzini et al. (2010b). The GTR+G and GTR+G substitution models were used in the ITS and LSU analyses, respectively. A partitioned matrix was used in all the analyses. Molecular-phylogenetic analyses were performed using the Bayesian Inference (BI) and Maximum Likelihood (ML) approaches. BI using Monte Carlo Markov Chains (MCMC) was carried out with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Four incrementally heated simultaneous MCMC were run over 5.000.000 generations, under model assumption. Trees were sampled every 500 generations resulting in an overall sampling of 10.001 trees. The "burn-in" value was evaluated using Tracer 1.5 (Rambaut & Drummond 2007). The first 20% of trees was discarded as "burn-in". For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). ML estimation was performed through RAxML v.7.0.4 (Stamatakis 2006) with 1.000 bootstrap replicates (Felsenstein 1985) using the

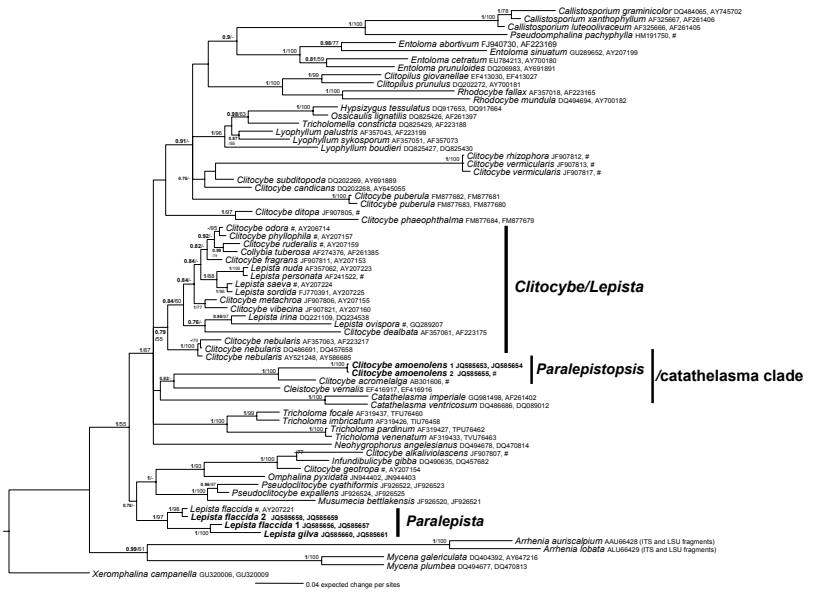
Callistosporium graminicolor DQ484065, AY745702
Callistosporium xanthophyllum AF325667, AF261406
Callistosporium luteoolivaceum AF325666, AF261405
Pseudoomphalina pachyphylla HM191750, #
Entoloma abortivum FJ940730, AF223169
Entoloma sinuatum GU289652, AY207199
Entoloma cetratum EU784213, AY700180
Entoloma prunuloides DQ206983, AY691891
Clitopilus giovanellae EF413030, EF413027
Clitopilus prunulus DQ202272, AY700181
Rhodocybe fallax AF357018, AF223165
Rhodocybe mundula DQ494694, AY700182
Hypsizygus tessulatus DQ917653, DQ917664
Osaliculis lignatilis DQ825426, AF261397
Tricholomella constricta DQ825429, AF223188
Lyophyllum palustris AF357043, AF223189
Lyophyllum sykosporum AF357051, AF357073
Lyophyllum boudieri DQ825427, DQ825430
Clitocybe rhizophora JF907812, #
Clitocybe vermicularis JF907813, #
Clitocybe vermicularis JF907817, #
Clitocybe subditopoda DQ202269, AY691889
Clitocybe candicans DQ202268, AY945055
Clitocybe puberula FM877682, FM877681
Clitocybe puberula FM877683, FM877680
Clitocybe ditopa JF907805, #
Clitocybe phaeophthalma FM877684, FM877679
Clitocybe odora #, AY206714
Clitocybe phyllophila #, AY207157
Clitocybe ruderalis #, AY207159
Collybia tuberosa AF274376, AF261385
Clitocybe fragrans JF907811, AY207153
Lepista nuda AF357062, AY207223
Lepista personata AF241522, #
Lepista saeva #, AY207224
Lepista sordida FJ770391, AY207225
Clitocybe metachroa JF907806, AY207155
Clitocybe vibecina JF907821, AY207160
Lepista irina DQ221109, DQ224538
Lepista ovispora #, GQ289207
Clitocybe dealbata AF357061, AF223175
Clitocybe/Lepista
Clitocybe nebularis AF357063, AF223217
Clitocybe nebularis DQ486691, DQ457658
Clitocybe nebularis AY521248, AY586685
Clitocybe amoenolens 1 JQ585653, JQ585654
Clitocybe amoenolens 2 JQ585655, #
Clitocybe acromelalga AB301606, #
Paralepistopsis
/catathelasma clade
Cleistocybe vernalis EF416917, EF416916
Catathelasma imperiale GQ981498, AF261402
Catathelasma ventricosum DQ486686, DQ089012
Tricholoma focale AF319437, TFUT6460
Tricholoma imbricatum AF319426, TIU76458
Tricholoma pardinum AF319427, TPUT6462
Tricholoma venenatum AF319433, TVUT6463
Neohygrophorus angelesianus DQ494678, DQ470814
Clitocybe alkaliviolascens JF907807, #
Infundibulicybe gibba DQ490635, DQ457682
Clitocybe geotropa #, AY207154
Omphaliina pyxidata JN944402, JN944403
Pseudoclitocybe cyathiformis JF926522, JF926523
Pseudoclitocybe expallens JF926524, JF926525
Musumecia bettlakensis JF926520, JF926521
Lepista flaccida #, AY207221
Lepista flaccida 2 JQ585658, JQ585659
Lepista flaccida 1 JQ585656, JQ585657
Lepista gilva JQ585660, JQ585661
Paralepista
Arrhenia auriscalpium kAU66428 (ITS and LSU fragments)
Arrhenia lobata ALU66429 (ITS and LSU fragments)
Mycena galericulata DQ404392, AY647216
Mycena plumbea DQ494677, DQ470813
Xeromphalina campanella GU320006, GU320009
0.04 expected change per sites

Figure 1. Tricholomatoid clade. Bayesian phylogram obtained from the combined ITS-LSU sequence alignment. Support values for clades that are supported in either the Bayesian (Posterior Probabilities values – BPP) or Maximum likelihood (ML Bootstrap percentage – MLB) analyses are indicated. BPP > 0.70 and MLB > 50% are given above branches. Numbers (1, 2) refer to the *Paralepistopsis* and *Paralepista* collections reported in Table 1.

GTRGAMMA algorithm for both ITS and LSU to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the "-f a" option of RAxML and "-x 12345" as a random seed to invoke the novel rapid bootstrapping algorithm. Only BPP values over 0.70 and MLB over 50% are reported in the resulting tree (Fig. 1). Pairwise % identity values of ITS sequences were calculated using MEGA 5.0 (Tamura et al. 2011).

## Results

### Molecular results

The combined dataset comprises a total of 71 taxa (including 66 from GenBank) and is 2412 base pairs long. The ITS and LSU datasets are 796 and 1616 base pairs long, respectively. Topologies of the combined ITS and LSU Bayesian and Maximum Likelihood trees are congruent (Fig. 1).

In both analyses the two *Clitocybe amoenolens* collections (ITS pairwise identity value = 99.9%) clearly cluster with the *C. acromelalga* collection (BPP 1 and MLB 100%) in the /catathelasma clade, a monophyletic group

in the tricholomatoid clade (Matheny et al. 2006, Ammirati et al. 2007). The ITS pairwise identity value between the *C. acromelalga* and *C. amoenolens* sequences is 94.4%; accepting an intraspecific variability lower than 3% (Nilsson et al. 2008), *C. amoenolens* and *C. acromelalga* should be considered distinct species. *Cleistocybe vernalis* Ammirati et al. cluster sister to the *C. acromelalga*/*C. amoenolens* pair (BPP 0.82); *Catathelasma* Lovejoy is basal to this group with low BPP and MLB values.

*Clitocybe amoenolens* and *C. acromelalga* have no phylogenetic relationship with other *Clitocybe* species. Accordingly, we propose to establish for them a new genus, *Paralepistopsis*.

*Lepista flaccida* and *L. gilva* are not phylogenetically related to the other *Lepista* species.

## Taxonomy

***Paralepistopsis*** Vizzini, **gen. nov.**

MycoBank MB 564340

*A* Paralepista *differt sporis levis, haud subglobosis, atque praesentia acidi acromelalgici qua de causa venenatae species sunt. et in stuctura molecularis (ITS-spatiis internis transcriptis et LSU DNA).*

Type species — *Clitocybe amoenolens* Malençon

Etymology — named in reference to its resemblance to *Paralepista* species.

Basidiomata agaricoid (with distinct pileus, lamellae and stipe), resembling those of *Paralepista gilva*, veils absent, spore-print whitish to cream, basidiospores thin-walled, smooth, inamyloid and slightly cyanophilous, pileal surface a cutis of repent to interwoven, cylindrical hyphae, clamp-connections present, no sarcodimitic texture in any part of the basidioma. On the ground, never on wood.

***Paralepistopsis amoenolens*** (Malençon) Vizzini, **comb. nov.** Fig. 2

MycoBank MB 564341

≡ *Clitocybe amoenolens* Malençon, in Malençon & Bertault, Flore des champignons superieurs du Maroc 2 - Trav. Inst. Sci. Chérifien, Sér. Bot. Biol. Vég. 33: 141 (1975).

Selected descriptions — Malençon & Bertault (1975: 138–141); Moreau et al. (2001: 99–100, 101–103).

Selected iconography — Malençon & Bertault (1975: pl. 8); Poumarat & Neville (1993: 48); Martínez et al. (2010: fig. 2, p. 104).

Pileus (2–)3.5–7(–8) cm diam., fleshy, sub-elastic, hemispherical to convex at first, becoming plano-convex and applanate, finally plano-concave, at times broadly umbonate; margin narrowly inrolled and decurved at first, remaining inrolled for a long time, then expanding to become somewhat wavy, shortly sulcate-striate or corrugated; not hygrophanous, occasionally appearing

hygrophanous when water soaked; surface slightly viscid when moist, at first entirely whitely pruinose, then pubescent-pruinose only at margin, typically diffracted-scaly near the disc, sometimes corrugated, wrinkled and areolate, later subglabrous often with more or less concentrically arranged watery, drop-like spots, especially near margin (as many *Lepista* species); at first cream-beige coloured (Capucine Buff, Pale Ochraceous-Salmon, Pale Ochraceous-Buff), then pinkish beige to rusty orange (Ochraceous-Buff, Zinc Orange) towards the centre. Lamellae crowded to close (L = 40–48(–50), l = (0–)1–2(–3)), thick, interspersed with lamellulae, (2–)3–4(–5) mm broad, decurrent, easily separable from the pileus context, at times intervenose or forked towards the stipe, at first whitish then yellowish ochre (Ivory Yellow, Chamois) to pinkish beige (Pale Ochraceous-Salmon, Pale Ochraceous-Buff); edges even, entire, concolorous. Stipe (2.5–)3–3.5(–5) cm long, 0.7–1.3 cm thick, short, central or subexcentric, equal or with a somewhat enlarged base, straight or recurved, concolorous with the pileus or slightly paler, minutely white pruinose at apex, glabrous elsewhere, stuffed with white medulla, becoming hollow; the base often with copious whitish tomentum with adhering *Pinaceae* needles and woody debris. Context 4–11(–14) mm thick at disc, elastic, white in the pileus, whitish cream (Capucine Buff) in the stipe cortex, unchanging; taste mild, fungoid, subfarinaceous, slightly bitter-farinaceous after long mastication; odour strong, aromatic, floral, reminiscent of *Inocybe corydalina*, *Tricholoma caligatum*, *Lepista irina,* or *Entoloma ameides*. Spore print whitish to pale cream (Light Buff, Pale Pinkish Buff).

Basidiospores (3.8–)4.0–5.4(–5.6) × (2.3–)3.2–4.0(–4.3) µm (n = 120), on average 4.8 × 3.4 µm, Q = 1.3–1.7, Qm = 1.42, broadly ellipsoid, hyaline, thin-walled, smooth, slightly cyanophilous, inamyloid, non-dextrinoid, usually with only a single oil drop and a distinct truncated apiculus up to 0.7 µm long, mostly adhering together in tetrads in dried specimens. Basidia (25–)30–37(–38) × 5–6(–7) µm, cylindro-clavate, usually four-spored, occasionally two-spored, sterigmata up to 5 µm long. Hymenophoral trama regular in young stages, but subirregular in mature basidiomata, consisting of hyaline, elongated, cylindrical hyphae 4–6(–8) µm broad. Pleurocystidia absent. Lamella edges fertile, with rare, scattered cells, not well differentiated from basidia, 15–45(–60) × 2.5–5(–7) µm, cylindric to subfusiform or sublageniform, often curved and flexuous, sometimes forked at apex, hyaline, thin-walled. Pileipellis duplex: upper layer (suprapellis) a soon disappearing, slightly gelatinous thin cutis (10–30 µm thick), of cylindrical hyphae, 1–3 µm broad; lower layer (subpellis) (150–350 µm thick) composed of densely arranged parallel to slightly interwoven hyphae (4–6 µm in diam.), terminal elements scattered, erect and repent cylindrical to fusiform-lageniform, sometimes with short lateral outgrowths. Pileitrama consisting of cylindrical or slightly

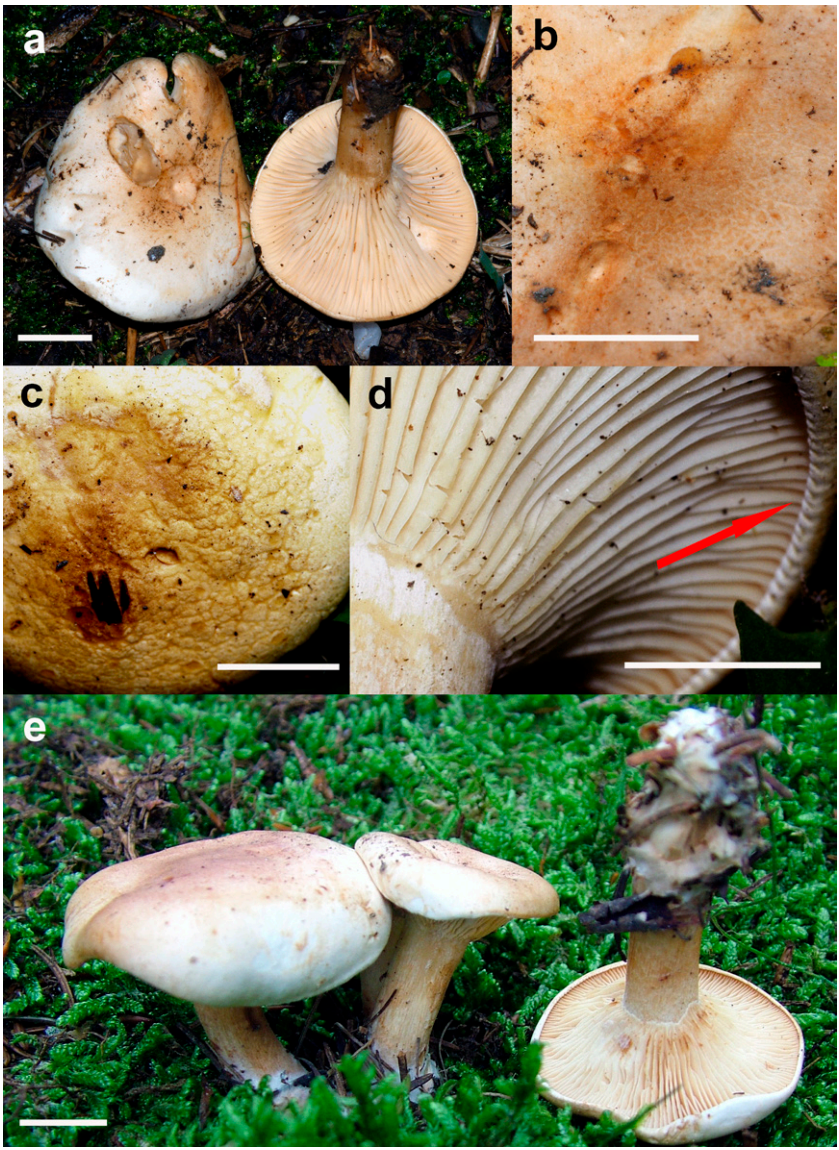


Figure 2. *Paralepistopsis amoenolens*. a. Basidiomata. b. Diffracted-scaly pileus with minute squamules. c. Areolate-corrugated pileus. d. Sulcate-striate pileus margin (red arrow), lamellae, and stipe. e. Basidiomata. [a–b from TO AV2007; c–d from TO AV2006 ; e from TO AV2004. Bars = 1 cm]

inflated, smooth hyphae, (4–)5–9(–12) µm broad, irregularly arranged, and with aeriferous lacunae. Stipitipellis a cutis consisting of hyphae 3–4 µm broad; towards the stipe apex with numerous thin-walled, flexuous, cylindrical to subfusiform caulocystidioid elements, 20–35 × 3–6 µm. Stipititrama formed by hyphae 6–7 µm broad. Thromboplerous hyphae (= oleiferous hyphae sensu Clémençon 2004) present especially in subpellis, pileus and hymenophoral trama. Pigment (yellowish) usually parietal and intracellular (cytoplasmatic) in the pileipellis; sometimes minutely incrusting or forming extracellular masses and granules. Clamp connections present at nearly all septa.

Habitat & distribution: scattered, gregarious, occasionally subcaespitose on pinaceous needle-beds and debris, often together with *Lepista flaccida*, on calcareous soil. Autumn. Very rare; known only from Morocco, France, Spain, and Italy.

material studied: **FRANCE**, **Savoie**, Haute-Maurienne valley, Lanslebourg-Mont-Cenis, 02 Sept 2011, litter of *Picea abies*, 1400 m asl, leg. G. Moretto (TO AV2004); 14 Oct 2011, leg. G. Moretto (TO AV2005). – **ITALY**, **Piedmont**, High Susa Valley, Salbertrand, Parco Naturale del Gran Bosco di Salbertrand, 20 Oct 2011, litter of *Picea abies*, 1500 m asl, leg. A. Vizzini (TO AV2006); Sauze d'Oulx, Parco Naturale del Gran Bosco di Salbertrand, 12 Nov 2011, litter of *Larix decidua*, 1550 m asl, leg. S. Anselmino (TO AV2007).

*Clitocybe amoenolens* was originally described from Morocco, in the Middle Atlas, growing among *Cedrus atlantica* litter in a high-altitude cedar forest (1600–1700 m a.s.l.) mixed with *Ilex aquifolium* and *Quercus ilex*, on calcareous soil (Malençon & Bertault 1975). It has recently been found in southern France (Bon 1987, Poumarat & Neville 1993) and in the Maurienne Valley (Charignon & Garcin 1998, Fourré 1997) in coniferous forests (*Pinus sylvestris*, *Larix decidua*, *Picea abies*) and always on calcareous soil.

*Clitocybe amoenolens* has been responsible for several erythromelalgic-type poisonings in the Maurienne Valley (Savoie, France) (Moreau et al. 2001, Saviuc & Danel 2006). It also has been found in the Abruzzi region (Centre Italy) under *Pinus nigra* (Contu et al. 1999), *P. nigra,* and *Cedrus* spp. (Leonardi et al. 2002) and *P. nigra* and *Larix decidua* (Leonardi & Maggi 2007), and poisoning cases referable to *C. amoenolens* have been recognised in this region (Leonardi et al. 2002, Marinetti & Recchia 2005). Finally, the species has been reported from Spain (Martínez et al. 2010), where it was collected in the autonomous regions of La Rioja (*Picea abies*) and Castilla-La Mancha (*Pinus pinaster* and *Cupressus arizonica* or *P. nigra* and *Quercus petraea*), close to the north and centre of Spain, respectively.

Our collections are the first record from northern Italy. According to our observations and bibliographic data, this species seems strictly restricted to *Pinaceae* in higher altitude thermophilic forests on calcareous soils.

***Paralepistopsis acromelalga*** (Ichimura) Vizzini, **comb. nov.**

MycoBank MB564342

≡ *Clitocybe acromelalga* Ichimura, Bot. Gaz. (Tokyo) 65: 110 (1918).

Selected descriptions — Ichimura (1918: 110); Moreau et al. (2001: 109–111).

Selected iconography — Guez (2010: (http://www.mycodb.fr/fiche.php?genre=Clitocybe&espece=acromelalga ).

Habitat & distribution: *Clitocybe acromelalga,* described from Japan (Ichimura 1918, Imazeki & Hongo 1957, Imazeki et al. 1988; Romagnesi 1989, Guez 1990), also occurs in South Korea (Lee & Hong 1985). It was reported as growing on both angiosperm (*Phyllostachys bambusoides, Acer palmatum*, *Zelkova serrata*) and gymnosperm (*Cryptomeria japonica*) litter.

**Phylogeny and specific delimitation**

In our combined ITS-LSU phylogenetic tree (Fig. 1) *Clitocybe amoenolens* and its sister *C. acromelalga* are not closely related to *C. nebularis* (Batsch) P. Kumm., the type of the genus *Clitocybe* (Redhead et al. 2002), nor to other *Clitocybe* species or allied taxa. As these two species represent a new phyletic line of clitocyboid fungi, it seems most appropriate to transfer them to the new genus *Paralepistopsis*.

Based on its habit, coloured lamellae and small spores, Bon (1997) and Moreau et al. (2001) placed *C. amoenolens* traditionally in subg. *Clitocybe* sect. *Gilvaoideae* Harmaja, where it occupies an isolated position. Contu et al. (1999), focusing on hymenial features (basidia longer than 30 µm), placed it in subgen. *Hygroclitocybe* Bon sect. *Clavipedes* Harmaja, a subgenus shown in recent molecular analyses (Redhead et al. 2002, Vizzini et al. 2011) to be artificial and heterogeneous.

The traditionally defined *Lepista* (Fr.) W.G. Sm. —clitocyboid fungi with a pinkish yellow spore print, usually separable lamellae, and inamyloid cyanophilous ornamented [verruculose to spiny] basidiospores (Singer 1986, Bon 1997, Consiglio & Contu 2003)— is a polyphyletic genus (Fig. 1). The species of *Lepista* subg. *Paralepista* (= *Lepista* sect. *Gilva*), which combine very crowded decurrent lamellae with subglobose to largely ellipsoidal spores, are not closely related either to *Lepista* s.s. or to other taxa in the tricholomatoid clade. Consequently we accept this lineage as a distinct genus and propose *Paralepista* for *Lepista flaccida*, *L. gilva* and allies. Following Bigelow (1985), Bon (1991), and Raithelhuber (2004), we list below all the taxa accepted in *Paralepista*:

***Paralepista*** Raithelh., Gattung *Clitocybe* 1: 17 (1981).

Type species — *Agaricus inversus* Scop.

≡ *Lepista* subg. *Paralepista* (Raitelh.) Bon, Doc. Mycol. 26(102): 18 (1996).

= *Clitocybe* sect. *Eulepistae* Singer, Ann. Mycol. 41: 40 (1943).

= *Lepista* sect. *Gilva* Harmaja, Karstenia 18: 53 (1978).
"*Lepista* sect. *Eulepista*" Konrad & Maubl., Icon. Select. Fung. 6(10): 350 (1936), nom. inval.
"*Lepista* sect. *Inversae*" Singer & Clémençon, Nova Hedwigia 23: 310 (1973 ["1972"]), nom. inval.

***Paralepista abdita*** (Dörfelt) Vizzini, **comb. nov.**
MycoBank MB 564343
≡ *Lepista abdita* Dörfelt, Boletus 1(2): 37 (1997).

***Paralepista ameliae*** (Arcang.) Vizzini, **comb. nov.**
MycoBank MB 564344
≡ *Clitocybe spinulosa* var. *ameliae* Arcang., Nuovo Giorn. Bot. Ital. 21: 434 (1889).

***Paralepista biformis*** (Peck) Vizzini, **comb. nov.**
MycoBank MB 564345
≡ *Clitocybe biformis* Peck, Bull. N.Y. St. Mus. 150: 25 (1911).

***Paralepista concentrica*** Raithelh., Metrodiana 23: 122 (1996).

***Paralepista femoralis*** (H.E. Bigelow) Vizzini, **comb. nov.**
MycoBank MB 564346
≡ *Clitocybe femoralis* H.E. Bigelow, Sydowia 36: 14 (1983).

***Paralepista flaccida*** (Sowerby) Vizzini, **comb. nov.**
MycoBank MB 564347
≡ *Agaricus flaccidus* Sowerby, Col. Fig. Engl. Fungi 2: pl. 185 (1799).

***Paralepista flaccida*** var. ***fibrillosa*** (Malençon) Vizzini, **comb. nov.**
MycoBank MB 564348
≡ *Clitocybe flaccida* var. *fibrillosa* Malençon, in Malençon & Bertault, Flore des champignons superieurs du Maroc 2 - Trav. Inst. Sci. Chérifien, Sér. Bot. Biol. Vég. 33: 157 (1975).

***Paralepista gilva*** (Pers.) Vizzini, **comb. nov.**
MycoBank MB 564349
≡ *Agaricus gilvus* Pers., Syn. Meth. Fung.: 448 (1801).
"*Paralepista gilva*" Raithelh., Metrodiana 23: 117 (1996), nom. inval.

***Paralepista inversa*** (Scop.) Raithelh., Gattung *Clitocybe* 1: 17 (1981).
≡ *Agaricus inversus* Scop., Fl. Carniol., Ed. 2, 2: 445 (1772).

***Paralepista lentiginosa*** (Fr.) Vizzini, **comb. nov.**
MycoBank MB 564350
≡ *Agaricus lentiginosus* Fr., Epicr. Syst. Mycol.: 69 (1838).

***Paralepista maculosa*** (Sacc.) Vizzini, **comb. nov.**
MycoBank MB 564352
≡ *Agaricus maculosus* Peck, Bull. Buffalo Soc. Nat. Sci. 1: 45 (1873), nom. illegit., non Pers. (1801).
≡ *Clitocybe maculosa* Sacc., Syll. Fung. 5: 183 (1887).

***Paralepista pseudoparilis*** (Enderle & Contu) Vizzini, **comb. nov.**
MycoBank MB 564353
≡ *Lepista pseudoparilis* Enderle & Contu, Beitr. Kenn. Pilze Mittel. 13: 12 (2000).

***Paralepista repanda*** (Raithelh.) Raithelh., Metrodiana 23: 121 (1996).
≡ *Lepista repanda* Raithelh., Metrodiana 14: 21 (1986 ["1985"]).

***Paralepista shafferi*** (H.E. Bigelow) Vizzini, **comb. nov.**
MycoBank MB 564354
≡ *Clitocybe shafferi* H.E. Bigelow, Beih. Nova Hedwigia 81: 339 (1985).

***Paralepista splendens*** (Pers.) Vizzini, **comb. nov.**
MycoBank MB 564355
≡ *Agaricus splendens* Pers., Syn. Meth. Fung.: 452 (1801).

## Discussion

*Paralepistopsis* species are characterized by a habit (decurrent and crowded lamellae) and colours (ochre-orange tinges) reminiscent of *Paralepista* or *Infundibulicybe* Harmaja, a whitish to cream spore print, smooth cyanophilic spores often arranged in tetrads in dried specimens and rarely exceeding 5(–6) µm in length. *Paralepista* differs in having strongly ornamented spores (Raithelhuber 1995, 2004); *Infundibulicybe* is distinguished by smooth lacrymoid spores with confluent bases and cyanophobic spore walls (Harmaja 2003).

*Paralepistopsis* clusters with *Cleistocybe* Ammirati et al. and *Catathelasma* in the /*catathelasma* clade. Because of the low resolution and lack of BPP and MLB support within the tree, a more precise, accurate position for the new genus could not be suggested. The presence of decurrent lamellae, confluent pileus and stipe, pale reddish brown colouration, and growth on soil are characters shared by *Paralepistopsis*, *Cleistocybe,* and *Catathelasma. Cleistocybe* and *Catathelasma* are distinguished from *Paralepistopsis* mainly by a partial veil, divergent to interwoven hymenophoral trama, and larger cyanophobic spores (Ammirati et al. 2007, Vizzini 2009); in addition *Catathelasma* spores are amyloid (Singer 1986).

*Paralepistopsis amoenolens* is delimited by a unique combination of macro-/micromorphological and chemical features, such as i) a strong aromatic, floral odour reminiscent of *Tricholoma caligatum*, *Inocybe corydalina, Lepista irina,* and *Entoloma ameides* caused by volatile metabolites identified by Fons et al. (2006) as methyl-(E)-cinnamate (also a key odorant of *T. caligatum*), methyl-benzoate, (E)-nerolidol, and methylanthranilate; ii) lamellae easily separating from the pileus context; iii) a cream coloured spore print; iv) smooth cyanophilic spores often arranged in tetrads; v) basidia reaching 35–40 µm; vi) pileipellis hyphae with short diverticula; vii) abundant thromboplerous hyphae; viii) and the presence of the toxic metabolite, acromelic acid A, a powerful neurotoxic

amino acid responsible for erythromelalgic poisoning and structurally homologous with kainic acid (a strong agonist of non-*N*-methyl-*D*-aspartate glutamate receptor subtypes) and domoic acid (Bessard et al. 2004).

*Paralepistopsis acromelalga* differs from *P. amoenolens* morphologically in a darker pileus and stipe, a pileus that soon becomes depressed, more crowded lamellae, a different odour, thromboplerous hyphae occurring only rarely, smaller spores (Ichimura 1918, Romagnesi 1989, Guez 1990, Miyauchi 1998, Moreau et al. 2001), and a more complex metabolite pattern (presence of acromelic acids A–E with 19 other toxins; Konno et al. 1983, 1988; Fushiya et al. 1990, 1992; Saviuc & Danel 2006). Additionally, our analyses show only a 91% pairwise ITS sequence identity between *P. acromelalga* and *P. amoenolens*. Singer (1986) transferred *P. acromelalga* to the heterogeneous genus *Neoclitocybe* Singer based on the presence of rare diverticulate hyphae in the pileipellis.

Based on their small spores and the *Paralepista*-like habit, *C. gilvaoides* Kauffman and *C. gracilis* (H.E. Bigelow & A.H. Sm.) Harmaja (sect. *Gilvaoideae*) from the coniferous forests of North America and Scandinavia (Bigelow 1985, Harmaja 1969) may also belong to *Paralepistopsis*, but more recently collected specimens are needed to perform molecular and biochemical analyses.

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**Literature cited**

Ammirati JF, Parker AD, Matheny PB. 2007. *Cleistocybe*, a new genus of *Agaricales*. Mycoscience 48: 282–289. http://dx.doi.org/10.1007/s10267-007-0365-5

Bas C. 1990. *Tricholomataceae* R. Heim ex Pouz. 65–70, in: C Bas et al. (eds). Flora Agaricina Neerlandica 2. A.A. Balkema, Rotterdam.

Bessard J, Saviuc P, Chane-Yene Y, Monnet S, Bessard G. 2004. Mass spectrometric determination of acromelic acid A from a new poisonous mushroom: *Clitocybe amoenolens*. J. Chromatogr. A. 1055: 99–107. http://dx.doi.org/10.1016/j.chroma.2004.08.133

Bigelow HE. 1985. North American species of *Clitocybe*. Part II. Beih. Nova Hedwigia 81: 281–471.

Binder M, Larsson K.-H, Matheny PB, Hibbett DS. 2010. *Amylocorticiales* ord. nov. and *Jaapiales* ord. nov.: early diverging clades of *Agaricomycetidae* were dominated by corticioid forms. Mycologia 102: 865–880. http://dx.doi.org/10.3852/09-288

Bon M. 1987. Quelques espèces intéressantes étudiées au stage FMDS de Saint Germain Monts d'Or. Bull. Féd. Mycol. Dauphiné-Savoie 105: 28–30.

Bon M. 1997. Les clitocybes, omphales et ressemblants. Doc. Mycol., mémoires hors-série 4: l–174.

Charignon Y, Garcin R. 1998. Un nouveau champignon toxique en France. Bull. Féd. Mycol. Dauphiné-Savoie 149: 11–14.

Clémençon H. 2004. Cytology and plectology of the *Hymenomycetes*. Bibl. Mycol. 199: 1–488.
Consiglio G, Contu M. 2003. Il genere *Lepista* in Italia. Riv. Micol. 46: 131–176.
Contu M, Signorello P, Anastase A. 1999. *Clitocybe amoenolens* Mal. in Abruzzo con osservazioni sulla sua posizione sistematica. Micol. Veget. Medit. 48: 16–18.
Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A. 2010. Geneious v5.3. Available from http://www.geneious.com/.
Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. http://dx.doi.org/10.2307/2408678
Fons F, Rapior S, Fruchier A, Saviuc P, Bessiere J. 2006. Volatile composition of *Clitocybe amoenolens*, *Tricholoma caligatum* and *Hebeloma radicosum*. Cryptogam. Mycol. 27: 45–55.
Fourré G. 1997. Intoxications: un sosie du *Lepista inversa* a produit en Savoie un terrifiant syndrome…japonais. Bulletin de la Société Mycologique du Massif d'Argenson 16: 6–11.
Fushiya S, Sato S, Kazasawa T, Kusano G, Nozoe S. 1990. Acromelic acid C. A new toxic constituent of *Clitocybe acromelalga*: a efficient isolation of acromelic acids. Tetrahedron Lett. 31: 3901–3904. http://dx.doi.org/10.1016/S0040-4039(00)97501-4
Fushiya S, Sato S, Kera Y, Nozoe S. 1992. Isolation of acromelic acids D and E from *Clitocybe acromelalga*. Heterocycles 34: 1277–1280. http://dx.doi.org/10.3987/COM-92-6065
Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113–118. http://dx.doi.org/10.1111/j.1365-294X.1993.tb00005.x
Guez D. 1990. Aperçu sur la flore mycologique du Japon. Bull. Féd. Mycol. Dauphiné-Savoie 116: 12–16.
Harmaja H. 1969. The genus *Clitocybe* (*Agaricales*) in Fennoscandia. Karstenia 10: 5–168.
Harmaja H. 2003. Notes on *Clitocybe* s. lato (*Agaricales*). Ann. Bot. Fenn. 40: 213–218.
Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17: 754–755. http://dx.doi.org/10.1093/bioinformatics/17.8.754
Ichimura T. 1918. A new poisonous mushroom. Bot. Gaz. (Tokyo) 65: 109–111. http://dx.doi.org/10.1086/332195
Imazeki R, Hongo T. 1957. Coloured illustrations of mushrooms of Japan, vol. I. Hoikusha Publ., Osaka.
Imazeki R, Otani Y, Hongo T. 1988. Fungi of Japan. Yama-Kei publ., Tokyo.
Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl. Acids Res. 30: 3059–3066. http://dx.doi.org/10.1093/nar/gkf436
Konno K, Shirahama H, Matsumoto T. 1983. Isolation and structure of acromelic acid A and B. New kainoids of *Clitocybe acromelalga*. Tetrahedron Lett. 24: 939–942. http://dx.doi.org/10.1016/S0040-4039(00)81571-3
Konno K, Hashimoto K, Ohfune Y, Shirahama H, Matsumoto T. 1988. Acromelic acids A and B. Potent neuroexcitatory amino acids isolated from *Clitocybe acromelalga*. J. Am. Chem. Soc. 110: 4807–4815. http://dx.doi.org/10.1021/ja00222a044
Kühner R. 1980. Les Hyménomycètes agaricoïdes. Bull. Soc. Linn. Lyon 49, num. spéc.:1–1027.
Lee JY, Hong SW. 1985. Illustrated flora and fauna of Korea. No. 28. Mushrooms. Ministry of Culture and Education, Korea.
Leonardi M, Maggi S. 2007. Nuova segnalazione per *Clitocybe amoenolens* in Abruzzo. Micol. Veget. Medit. 22: 80.
Leonardi M, Ciulli G, Pacioni G, Recchia G. 2002. Una intossicazione collettiva da *Clitocybe amoenolens* riconducibile alla sindrome acromelalgica. Micol. Veget. Medit. 17: 133–142.

Malençon G, Bertault R. 1975. Flore des champignons supérieurs du Maroc, tome 2. Trav. Inst. Sci. Chérifien, Sér. Bot. Biol. Vég. 33: 1–540.

Marinetti V, Recchia G. 2005. Nuovi casi di sindrome acromelalgica in Abruzzo. Boll. Gr. micol. G. Bres. (n.s.) 48: 39–43.

Martínez F, Martínez R, Meléndez A, Pérez Del Amo CM. 2010. *Clitocybe amoenolens*. Primera cita para España. Bol. Soc. Micol. Madrid 34: 103–112.

Matheny PB, Curtis JC, Hofstetter V, Aime MC, Moncalvo JM, Ge ZW, Yang ZL, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjarden DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS. 2006. Major clades of *Agaricales*: a multi-locus phylogenetic overview. Mycologia 98: 982–995. http://dx.doi.org/10.3852/mycologia.98.6.982

Miyauchi S. 1998. Comparison *Clitocybe acromelalga* with *Clitocybe* sp. collected in France. Rapport of the Nagaoka University of Technology, sect. Bio-Ingeneering Kamitomioka 1603-1, Nagaoka, Japan.

Moncalvo J-M., Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Clémençon H, Miller OK Jr. 2002. One hundred and seventeen clades of euagarics. Mol. Phylogenet. Evol. 23: 357–400. http://dx.doi.org/10.1016/S1055-7903(02)00027-1

Moreau PA, Courtecuisse R, Guez D, Garcin R, Neville P, Saviuc P, Seigle-Murandi F. 2001. Étude taxinomique d'une espèce toxique: *Clitocybe amoenolens* Malençon. Cryptogam. Mycol. 22: 95–117. http://dx.doi.org/10.1016/S0181-1584(01)80003-8

Nakamura K, Shoyama F, Toyama J, Tateishi K. 1987. Empoisonnement par le Dokou-sassa-ko (*Clitocybe acromelalga*). Jpn. J. Toxicol. 1: 35–39.

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson K-H. 2008. Intraspecific ITS variability in the Kingdom Fungi as expressed in the International Sequence Databases and its implications for molecular species identification. Evol. Bioinf. 4: 193–201.

Poumarat S, Neville P. 1993. Espèce de la zone du *Quercus ilex* au Maroc, montagnarde en France, *Clitocybe amoenolens* Malençon. Bull. Fédér. Assoc. Mycol. Médit. 4: 16–19.

Redhead SA, Lutzoni F, Moncalvo JM, Vilgalys R. 2002. Phylogeny of agarics: partial systematics solutions for core omphalinoid genera in the *Agaricales* (Euagarics). Mycotaxon 83: 19–57.

Romagnesi H. 1989. Curiosité mycologique: un champignon tortionnaire japonais: *Clitocybe acromelalga* Ichimura. Bull. Soc. Mycol. France 105: 131–132.

Raithelhuber J. 1995. Trichterlinge mitteleuropas. Metrodiana 22(2): 52–94.

Raithelhuber J. 2004. Mitteleuropäische Trichterlinge. Gattungen *Clitocybe*, *Pseudolyophyllum* und *Paralepista*. JH. Raithelhuber Außenseiterverlag, Stuttgart.

Rambaut A, Drummond AJ. 2007. Tracer v1.4. [Available from http://beast.bio.ed.ac.uk/Tracer].

Ridgway R. 1912. Color standards and color nomenclature. Washington, D.C., published privately (by the author). 43 p. + 53 color pls.

Saviuc PF, Danel VC, Moreau PA, Guez DR, Claustre AM, Carpentier PH, Mallaret MP, Ducluzeau R. 2001. Erythromelalgia and mushroom poisoning. Clin. Toxicol. 39: 403–407. http://dx.doi.org/10.1081/CLT-100105162

Saviuc PF, Danel VC, Moreau PA, Claustre AM, Ducluzeau R, Carpentier PH. 2002. Érythermalgie soudaine: cherchez le champignon! Rev. Méd. Interne 23: 394–399. http://dx.doi.org/10.1016/S0248-8663(02)00576-3

Saviuc PF, Danel VC. 2006. New syndromes in mushroom poisoning. Toxicol. Rev. 25: 199–209. http://dx.doi.org/10.2165/00139709-200625030-00004

Singer R. 1986. The *Agaricales* in modern taxonomy, 4th ed. Koeltz Scientific Books, Koenigstein.

Stamatakis A. 2006. RAxML-VI-HPC: Maximum Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. http://dx.doi.org/10.1093/bioinformatics/btl446

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28: 2731–2739. http://dx.doi.org/10.1093/molbev/msr121

Thiers B. 2011. (continuously updated). Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol. 172: 4238–4246.

Vizzini A. 2009. Due nuove combinazioni nel genere *Cleistocybe* (*Basidiomycota, Agaricomycetes*), con validazione di *Hygrophorus pleurotoides*. Micol. Veget. Medit. 24: 95–98.

Vizzini A, Musumeci E, Murat C. 2010a. *Trichocybe*, a new genus for *Clitocybe puberula* (*Agaricomycetes*, *Agaricales*). 2010. Fung. Diver. 42: 97–105. http://dx.doi.org/10.1007/s13225-010-0030-8

Vizzini A, Contu, M., Musumeci, E. and Ercole E. 2010b. A new taxon in the *Infundibulicybe gibba* complex (*Basidiomycota*, *Agaricales*, *Tricholomataceae*) from Sardinia (Italy). Mycologia 103: 203–208. http://dx.doi.org/10.3852/10-137

Vizzini A, Contu M., Ercole E. 2011. *Musumecia* gen. nov. in the Tricholomatoid clade (*Basidiomycota*, *Agaricales*) related to *Pseudoclitocybe*. Nord. J. Bot., Article first published online: 20 OCT 2011. http://dx.doi.org/10.1111/j.1756-1051.2011.01169.x

White TJ, Bruns TD, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR Protocols. Academic Press, London.